

IN THE SPECIFICATION:

Please amend paragraph [0030] as follows:

--[0030] In one embodiment, an isolated polypeptide comprising the amino acid sequence Y (Trp/Phe) Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ (Trp/Phe) Xaa₆ Xaa₇ (Trp/Phe) Z (SEQ ID NOs: 17-20) is provided. Y, which may or may not be present, is a peptidic structure containing at least one cysteine residue and having the formula (Xaa)_n. Xaa is any amino acid residue and n is an integer from 1 to 20. Z, which may or may not be present, is a peptidic structure containing at least one cysteine residue and having the formula (Xaa)_n, wherein Xaa is any amino acid residue and n is an integer from 1 to 20. The amino acid residues of in Xaa₁ through Xaa₇ can be any amino acid and the amino acid residues of Xaa₁ through Xaa₅ are positively charged. --

Please amend paragraph [0031] as follows:

--[0031] In another embodiment, an isolated polypeptide comprising the amino acid sequence Y (Trp/Phe) Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ (Trp/Phe) Xaa₆ Xaa₇ Xaa₈(Trp/Phe) Z (SEQ ID NOs: 21-24) is provided. Y, which may or may not be present, is a peptidic structure containing at least one cysteine residue and having the formula (Xaa)_n. Xaa is any amino acid residue and n is an integer from 1 to 20. Z, which may or may not be present, is a peptidic structure containing at least one cysteine residue and having the formula (Xaa)_n, wherein Xaa is any amino acid residue and n is an integer from 1 to 20. The amino acid residues of Xaa₁ through Xaa.sub.8 is any amino acid, and at least two of the amino acid residues of Xaa₁ through Xaa₅ are positively charged. --

Please amend paragraph [0059] as follows:

--[0059] To verify that the DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) peptide would identify A β ₁₋₄₀ amyloid independently of its presence in bacteriophage, this peptide was produced in recombinant form as a fusion protein with thioredoxin. Cysteines were engineered at either end of the peptide in the fusion construct, along with several other residues from the phage coat protein. The ultimate or penultimate residue was engineered to be a proline to mimic predicted beta turn at either side of

the 20 amino acid insert. This protein was termed Thio-A β . Recombinant thioredoxin without the A β -peptide binding sequence was termed Thio. --

Please amend paragraph [0061] as follows:

--[0061] Chemically synthesized peptides were tested to identify binding to A β ₁₋₄₀ amyloid (FIG. 5). To test the relative contribution of the flanking (N-terminal and C-terminal) cysteines, peptides were synthesized that either had or did not have these residues; Both peptides were made with an N-terminal biotin label to allow identification using streptavidin. Biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) and Biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4) were tested for binding to amyloid A β ₁₋₄₀. Biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4) bound A β ₁₋₄₀ amyloid with a K_d of 320 nM. Biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2), which lacks flanking cysteines, showed binding in the 10-80 μ M range. These data indicate that the terminal cysteines, which can form a disulfide bond, induce a conformation of the 20 amino acid insert that enhances high affinity binding to A β ₁₋₄₀ amyloid. --

Please amend paragraph [0062] as follows:

-- [0062] Staining of AD and non-AD brain was repeated using Biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4) (FIG. 6). In addition, tissue from organs (kidney, brain, large bowel, small bowel, and prostate) from a non-AD patient with amyloidosis were stained. As with Thio-A β , the synthetic peptide specifically stained amyloid plaques in AD brain. No staining of neurofibrillary tangles was detected. As with binding to A β ₁₋₄₀ amyloid in vitro, slightly higher concentrations were needed for staining of plaques relative to Thio-A β . Concentrations as low as 500 nM gave good staining with the peptide. Simultaneous staining of kidney sections containing non-A β amyloid demonstrated that binding was specific for A β amyloid. Thus, this peptide sequence is a high affinity probe for A β amyloid both in vitro and in vivo, both within and outside the context of other recombinant protein sequences.--

Please amend paragraph [0063] as follows:

--[0063] Using phage display, at least two cysteine-linked 20 amino acid peptide sequences that bind to the amyloid form of A β ₁₋₄₀ have been identified. Neither of these sequences bind monomeric A β ₁₋₄₀, and therefore these peptides specifically identify the amyloid form of the A β ₁₋₄₀ protein. Both of these sequences share a [(W/F)X₅(W/F) X_{2/3} (W/F)] (SEQ ID NOs: 1 and 33, respectively) structure in common, and both have two positively charged (and no negatively charged) amino acids within the X₅ region. Therefore, cysteine-linked peptides with this repeating hydrophobic motif provides a template for the design of other peptides that can bind to A β amyloid with even higher affinity. Production and purification of one of these peptide sequences as a fusion protein with thioredoxin (Thio-A β), or direct chemical synthesis of the peptide, created a high affinity binding protein for A β ₁₋₄₀ amyloid in vitro. These reagents also bound specifically to amyloid plaques in Alzheimer's disease (AD) brain.--

Please amend paragraph [0074] as follows:

-- [0074] Production of recombinant A.beta.-binding peptide: Complementary oligonucleotides encoding the DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) peptide sequence were annealed, digested with Kpn I and Xba I, and ligated into pThioHisC plasmid (Invitrogen; Carlsbad, Calif.) at the Kpn I and Xba I sites using the following sequences:--

Please amend paragraph [0078] as follows:

-- [0078] For binding to synthetic peptides, two peptides were synthesized and purified containing an N-terminal biotin. One of these was the 20 amino acid-insert without any flanking sequences, Biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2). The other sequence, Biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4), contained flanking cysteine residues and several other amino acids from the bacterial coat sequence. These peptides were purified by HPLC and confirmed by mass spectrometry to be over 90% pure. Peptides were solubilized in phage buffer and incubated at varying concentrations with A β ₁₋₄₀ amyloid for 1 hour. After washing in phage buffer as above, streptavidin conjugated to alkaline phosphatase

was added at 1 U/ml for 50 minutes. Plates or slides were washed extensively in phage buffer and developed, as above.--

Please amend paragraph [0082] as follows:

-- [0082] 100 µL of a 1 mg/mL solution of amyloid binding-peptide (biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4)) or control peptide without cysteines (biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)) was injected via the tail vein into 8-9 month old wild type CB6 mice. Peptides were injected in sterile Tris-buffered saline, pH 7.4, with 1 mM CaCl₂ and 1 mM MgCl₂. 4 animals were injected for each condition. Animals were then sacrificed after one minute or two minutes. Immediately upon sacrifice, blood and organs were harvested. To quantitate peptide uptake, brain, liver, and kidney were washed and the tissues lysed by homogenization in doubly distilled water. Organs and blood were centrifuged at 13000 g to collect lysate. Organ lysates or serum were then immobilized on ELISA plates that had been coated with nitrocellulose. Some tissue and serum samples were spiked with known amounts of purified peptide to verify levels of peptide immobilization in different sample types. For histochemical detection of biotinylated peptides in brain, a portion of the brain from each experiment was dissected and fixed for one day in 4% paraformaldehyde. These tissues were then dehydrated in PBS with varying levels of sucrose, frozen, and sectioned as described in Hoyte et al. (Brain Research: Molecular Brain Research 109:146-160 (2002)). 8 mm brain sections were stained for biotinylated peptide by binding of alkaline phosphatase-conjugated streptavidin. Levels of streptavidin binding were determined by development of sections in nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The levels of biotinylated peptide immobilized on ELISA plates was quantitated by binding of alkaline phosphatase-conjugated streptavidin. Levels of streptavidin binding were determined by development with para-nitrophenylphosphate, as in Kang et al. (Neurobiology of Disease 14:146-156 (2003)).--

Please amend paragraph [0087] as follows:

--[0087] FIG. 1 shows staining of amyloid A β ₁₋₄₀ by phage peptides. Both phage peptide sequences selected for A β ₁₋₄₀ amyloid binding stained amyloid deposits in vitro. Final clones 2 and 4 are the DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) sequence. This sequence identified both small and large (0.5-50 μ m) accumulations of A β ₁₋₄₀ amyloid. Final clone 6 is the PGRSPFTGKKLFNQEFSQDQ (SEQ ID NO:3) sequence. This sequence stained A β ₁₋₄₀ amyloid aggregates more poorly, but still stained well above background levels. None of the ten starting clones randomly picked (Starting clone 6 is shown) stained when used at the same concentration.--

Please amend paragraph [0089] as follows:

-- [0089] FIG. 3 shows recombinant A β -binding peptide binds with high affinity to A β ₁₋₄₀ amyloid in vitro. A recombinant cysteine-linked form of the DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) sequence was produced as a fusion protein with thioredoxin in E. coli (Thio-A β). Recombinant Thio-A β was purified and binding to A β ₁₋₄₀ amyloid was measured. Recombinant Thio-A β bound A β ₁₋₄₀ amyloid with a K_d of 60 nM. Binding was saturating by 200 nM. Recombinant purified thioredoxin (Thio) showed no binding at any of the concentrations used. Errors are SEM for n=6.--

Please amend paragraph [0091] as follows:

--[0091] FIG. 5 shows binding of synthetic peptides to A β ₁₋₄₀ amyloid in vitro. Two biotin-labeled peptides, Biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) and Biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4), were tested for binding to A β ₁₋₄₀ amyloid. The peptide containing flanking cysteines bound with a K_d of 320 nM, while the peptide lacking these cysteines did not bind with significant affinity below 5 μ M. Errors are SEM for n=6.--

Please amend paragraph [0094] as follows:

-- [0094] FIG. 8 depicts entry of amyloid binding peptide into the brain after intravenous injection. Amyloid binding peptide (biotin-AECDWGKGGRWRLWPGASGKTEACGP (SEQ ID NO:4)) was compared to

binding of a control peptide lacking cysteines (biotin-DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)) 2 minutes after intravenous injection via the tail vein in wild type mice. Control peptide was present in some blood vessels, but did not enter the brain parenchyma. Amyloid binding peptide, by contrast, entered the brain parenchyma in large amounts. Sagittal section of cortex is shown. The bar indicates 100 μ m in A, B, 50 μ m in C, D.--

Please amend paragraph [0095] as follows:

-- [0095] Table 1: Identification of peptides that adhere to A β ₁₋₄₀ amyloid. A random 20-amino acid cysteine-cross-linked phage peptide library with 5×10^7 possible sequences was screened for adhesion to A β ₁₋₄₀ amyloid. Sequences of 10 randomly picked phage clones in the starting library are shown, as are sequences of 10 randomly picked phage clones isolated after three rounds of panning against A β ₁₋₄₀ amyloid. At least two peptides adhered to A β ₁₋₄₀ amyloid. These sequences shared a density of similarly spaced bulky hydrophobic amino acids (underlined) that were not present in clones picked from the starting library. Two positively charged amino acids (dark) were present between the first two hydrophobic residues in both peptides.

Random Starting Clone Sequences:

1. LGSGRIGDGWSDGGLARRLK (SEQ ID NO:7)
2. DGGGGAGRWTTKDRSAAKTE (SEQ ID NO:8)
3. VDDGAQGKRWGGMGLGKGRR (SEQ ID NO:9)
4. SGSGVGLRMASQRHEGRKVY (SEQ ID NO:10)
5. QLPQNGGPAWFTRKAGQGGR (SEQ ID NO:11)
6. LGYAGGGQGMVEGSFWPTSW (SEQ ID NO:12)
7. GLRGMEGRGYPKDRRDRNLE (SEQ ID NO:13)
8. LIGGNKAGRGAWGVVASSGR (SEQ ID NO:14)
9. ELESRGGLGYAWRGSASTMD (SEQ ID NO:15)
10. KGETGNNGRAKAGTVDLIRR (SEQ ID NO:16)

Random Final Clone Sequences:

1. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
2. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
3. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
4. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
5. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
6. PGRSPFTGKKLFNQEFSDQ (SEQ ID NO:3)
7. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
8. PGRSPFTGKKLFNQEFSDQ (SEQ ID NO:3)
9. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2)
10. DWGKGGRWRLWPGASGKTEA (SEQ ID NO:2) –

Please amend paragraph [0096] as follows:

--[0096] Table 2: Rate of uptake of amyloid binding peptide in brain, kidney, and liver. The rate of uptake of amyloid binding peptide was quantitated as a percentage of the concentration delivered intravenously into serum and compared to that for a non-cysteine containing control peptide. Only the amyloid binding peptide containing cysteines was delivered into the brain at a significant rate, and was equivalent to the rate of uptake into the kidney or the liver. Errors are SD for n=4.

<u>Organ</u>	<u>Peptide (SEQ ID NO:)</u>	<u>% uptake/min</u>
Brain	biotin-AECDWGKGGRWRLWPGASGKTEACGP (<u>4</u>)	0.18 ± 0.02%
Liver	biotin-AECDWGKGGRWRLWPGASGKTEACGP (<u>4</u>)	0.06 ± 0.02%
Kidney	biotin-AECDWGKGGRWRLWPGASGKTEACGP (<u>4</u>)	0.16 ± 0.02%
Brain	biotin-DWGKGGRWRLWPGASGKTEA (<u>2</u>)	0.00005 ± 0.00005%

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.--

Please replace the previously filed sequence listing with the attached sequence listing. Please enter the accompanying sequence listing into the application.